

In the Claims

Please cancel claims 45-66 without prejudice and without disclaimer. New claims are found in the Listing of the Claims beginning on page 2.

Applicants' Remarks and Response to Final Office Action of April 30, 2004 are found beginning on page 5.

LISTING OF THE CLAIMS

1-66. (canceled)

67. (new) A method for cryopreservation of oocyte morphology and viability, comprising the steps:

(a) suspending oocytes in a medium comprising about 4% ethylene glycol and about 20% bovine serum for about 15 min at a temperature at or near physiological temperatures;

(b) rinsing the oocytes for about 30 seconds in a solution comprising about 35% ethylene glycol, a sugar, bovine serum and a macromolecule;

(c) vitrifying the oocytes in step (b) by quickly dropping microdroplets of the solution comprising the oocytes onto a solid surface that has a temperature between -150°C and -180°C ; and

(d) collecting frozen microdroplets that contain vitrified oocytes that maintain morphology and viability after thawing.

- 68. (new) The method of claim 67 wherein cleavage rates of oocytes after thawing and *in vitro* fertilization are about 84% of cleavage rate of non-vitrified oocytes.
- 69. (new) The method of claim 67 wherein blastocyst formation of oocytes after thawing and *in vitro* fertilization is about 58% of blastocyst formation of non-vitrified oocytes.
- 70. (new) The method of claim 67 wherein the suspending is at near physiological temperature of 39°C;
- 71. (new) The method of claim 67 wherein the rinsing is for about 25 seconds.
- 72. (new) The method of claim 67 wherein the solution in step (b) further comprises a surfactant
- 73. (new) The method of claim 67 wherein the macromolecule has a surfactant effect at room temperature.
- 74. (new) The method of claim 67 wherein the macromolecule is polyvinylpyrrolidone.
- 75. (new) The method of claim 67 wherein the sugar is trehalose.
- 76. (new) The method of claim 72 wherein the surfactant is bovine serum albumin, or fetal bovine serum.
- 77. (new) The method of claim 67 wherein the medium comprises a TCM 199 base medium.
- 78. (new) The method of claim 67 further comprising storing the collected frozen microdroplets for at least three weeks prior to thawing.

- 79. (new) The method of claim 67 further comprising partially or fully removing oocyte cumulus cells prior to step (a).
- 80. (new) The method of claim 67 wherein the ethylene glycol raises the vitreous state glass transition temperature in the microdroplets sufficiently to inhibit ice formation.
- 81. (new) The method of claim 67 further comprising thawing the vitrified oocyte microdroplets at a near physiological temperature for up to about 3 minutes.
- 82. (new) The method of claim 67 wherein the oocytes are non-human mammalian oocytes.
- 83. (new) The method of claim 81 further comprising fertilizing the thawed oocyte.
- 84. (new) The method of claim 83 wherein the fertilized thawed oocyte is incubated to form an embryo.
- 85. (new) The method of claim 84 wherein incubation is by cumulus cell-coculture with KSOM and fetal bovine serum media.
- 86. (new) The method of claim 67 wherein the microdroplets have a volume of about 1-10 microliters.
- 87. (new) The method of claim 86 wherein the microdroplets have a volume of 1 microliter.
- 88. (new) The method of claim 81 further comprising enucleating the thawed oocytes for somatic cell nuclear transfer.
- 89. (new) The method of claim 88 wherein the hatched blastocyst rate from nuclear transfer embryos is about 91% of rate of development from non-vitrified oocytes.